Final Report

Bioassessment Monitoring of Acid Mine Drainage Impacts in Streams of the Leviathan Mine Watershed: Update for Spring-Fall 2015 Surveys

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Summary for 2015:

Indicators of biological health of the stream invertebrate community in Bryant Creek continued to show stable recovery near or matching reference stream expectations. Closer to the mine, at Leviathan Creek above Mountaineer Creek, the community continues to approach reference state. Although sites nearest the mine on Leviathan and Aspen Creek show mixed signs of recovery, they remain in an impaired ecological state. Drought in 2015 reached record levels of low spring runoff, and higher densities of invertebrates were found than have occurred in average or wet years, as habitat area contracted and scouring flows did not occur. Leviathan Creek below mine (above Aspen confluence), was again dry by the September collection date, further stressing conditions at this location. Even though drying plagues this site along with AMD exposure, gains in biological health at other sites were sustained through years of intensifying drought.

Introduction - Background

The pollution of streams by runoff from mining excavations can damage aquatic life long after mines have ceased operation. Acidic water, toxic metals, and contaminated sediments can combine to make affected sections of streams nearly uninhabitable by native macroinvertebrates. Restoration of water and habitat quality often requires a variety of remedies applied over many years. Recovery of natural biological communities can be used to evaluate the success of remediation programs and benthic or bottom-dwelling invertebrates are often used for the purpose of judging changes in ecological health. The studies reported here apply benthic invertebrate bioassessment metrics for long-term monitoring of ecological recovery in the Leviathan Creek watershed. These monitoring data constitute one of the longest continuous bioassessment records of mine runoff impact and recovery on streams anywhere (1998-2015, ongoing).

Leviathan Mine is an abandoned open pit sulfur mine site located just north of Monitor Pass on Highway 89 in Alpine County, in the central Sierra Nevada of California. Covering an area of 650 acres (250 with visible mining disturbance), the mine last operated on a large scale in the 1950s and early 1960s, primarily for sulfur extraction. Acid mine drainage (AMD) from this site enters Leviathan Creek and Aspen Creek, flows 2.5 km from their confluence to become Bryant Creek where it joins with Mountaineer Creek, flowing a further 11 km where it enters the East Fork of the Carson River in Douglas County, Nevada. Acid drainage emanates from the following identified locations: the mine adit, mine pit underdrain (PUD), the Leviathan Creek channel underdrain (CUD), the Delta Seep, and Aspen Seep. Together these discharges contribute acid drainage containing a mixture of dissolved and particulate toxic metals, and orange ferric hydroxide precipitates ("yellow-boy") to Leviathan Creek. In May of 2000 the U.S. Environmental Protection Agency (EPA) listed Leviathan Mine as a Superfund (CERCLA) site to facilitate site remediation and coordinate planning activities.

Discharge from the Adit and PUD is contained in collecting ponds. These ponds overflowed during late winter and spring snow-melt periods until 2000. The CUD and Delta Seep discharge directly to Leviathan Creek. Aspen Seep discharges to Aspen Creek. Active seasonal chemical treatment of AMD sources began in earnest in the autumn of 1999 and has continued since, with the result that the ponds have seldom overflowed since the spring of 1999. Pond water is typically treated through lime addition in June-September (sometimes earlier when ponds are accessible), settled to remove precipitates and then discharged to Leviathan Creek after chemical testing. The CUD has also been intercepted and actively treated through lime addition during summer or early fall depending on weather conditions. The Delta Seep was partly or completely captured during the summers of 2003, 2004, and 2007 through 2015. Treatment of CUD and Delta Seep is discontinued and discharges are returned to Leviathan Creek at the conclusion of each field season. Aspen seep has been treated year around in a microbial bioreactor system since 1999. These actions have substantially reduced, but not eliminated the discharge of AMD to Leviathan Creek. During the period of 2004 and 2005, the most substantial changes in treatment regime were that in 2005 the CUD treatment period was shorter and capture of the Delta Seep was discontinued until 2007. Another source of acid seepage was from an off-channel marsh created by a landslide on Leviathan Creek just above the Leviathan above Aspen monitoring station (this was noted in 2008). In the summer of 2010 a beaver pond expanding this marsh on Leviathan

Creek was found and continues to pass through acid discharge from the mine site.

Bioassessment monitoring of aquatic invertebrates such as insects has been conducted since 1995 in streams of the Leviathan Creek watershed to provide an ecological evaluation of AMD effects on aquatic life and the progress of remediation. Benthic stream invertebrates are sensitive to chemical pollution and physical habitat disturbance and provide a useful indicator tool for assessment of biological integrity (Barbour et al. 1999, Rosenberg and Resh 1993). Aquatic macroinvertebrate bioassessment has been previously used to define the spatial extent of biological impacts in the Leviathan-Bryant Creek watershed in 1995, 1997, and 1998 through 2014, with most sampling also conducted in late spring and early fall of each year (June and September) and summarized in a series of report updates (Herbst 1995, 1997, 2000, 2002, 2004a, 2004b, 2007, 2009, 2011, 2012, 2013, 2014, 2015a, 2015b, 2016). These data have established the ongoing changes in condition of the benthic invertebrate community along downstream AMD-affected sites and in reference streams, and document seasonal and year-to-year variations. The objective of this report is to provide an update for spring and fall 2015 bioassessment monitoring at sites in Leviathan and Bryant Creeks exposed to acid drainage discharges and an interpretation of ecological recovery. This continues development of a data set for evaluating the progression of improving conditions over time or relapses in health, and for use as indicators of the re-establishment of aquatic life to a natural state.

A group of 8 sample stations were surveyed in June and 7 in September of 2015 as described below. The sample sites were located just below the mine on Aspen and Leviathan Creeks (Leviathan site dry in Fall 2015), on Leviathan Creek just above its confluence with Mountaineer Creek, on Mountaineer Creek just above confluence with Leviathan, on Bryant Creek below the confluence formed by Leviathan and Mountaineer, on Bryant Creek near the Stateline boundary, and upper Leviathan Creek above Aspen at a location above beaver ponds designated 4L where flows are sustained even though drying below (Figure 1). In addition to these sites, sampling stations usually include reference sites of similar size or setting, and in 2015 a reference site was again located on the upper portion of Mountaineer Creek. Reference site sampling over the years of monitoring AMD-exposed sites are intended to frame background conditions of similar streams to represent the range of potential invertebrate communities that could be expected to occur in Leviathan and Bryant Creeks. The seasonal sampling periods were selected to represent changing hydrologic conditions during spring run-off and fall base-flow, phenological changes in the development of insect populations, and near the beginning of the spring AMD treatments and end of operations in fall. Mountaineer Creek at its lower end just above joining Leviathan Creek, has served as the primary reference site for biomonitoring throughout the history of this survey program. To provide additional context for natural stream flow variation that may affect aquatic invertebrate populations, hydrographs through 2015 are shown for the East Fork Carson River (Figure 2), representing the larger watershed to which Leviathan, Mountaineer, and Bryant creeks are tributary, and for Bryant Creek below Mountaineer (Figure 3), to show local flow conditions in the Leviathan, Mountaineer, and Bryant drainages. Flows in 2015 reflect a fourth consecutive drought year, contrasting to 2011 conditions where deep snowpack produced prolonged high runoff conditions.

Bioassessment Monitoring and Methods

The purpose of the monitoring program described here is to provide biological measures of ecological health using various attributes of the stream invertebrate community as indicators. These data will assist managers in delineating the area impacted by AMD, and establish a status condition for continued monitoring of the extent and progress of chemical and ecological recovery of stream water quality and habitat. Biological structure and function of aquatic ecosystems are not always obvious features of the environment, so practical field techniques are needed to assess the ecological health of streams. Aquatic insects and other invertebrates are central to the function of stream ecosystems, consuming organic matter (wood and leaf debris) and algae, and providing food to higher trophic levels (fish and riparian birds). These native organisms also have varying degrees of pollution tolerance and so may be used as integrative indicators of water quality and habitat conditions. For example, distinctive shifts in the structure and function of the aquatic invertebrate community can often be detected between upstream and downstream of a pollution source. Such use of the stream invertebrate fauna in evaluating stream ecosystem health is known as bioassessment. The technique relies on collections of the benthos (bottom-dwelling fauna) to evaluate the relative abundance of different taxa, feeding guilds, pollution indicators, and biodiversity, in order to develop a quantitative basis for measuring ecological attributes of the stream. Monitoring relative to reference sites (having little or no impact but similar physical setting), and/or over time within subject sites, then permits impact problems or recovery to be quantified (Rosenberg and Resh 1993). Previous studies of AMD impacts on stream communities have also utilized macroinvertebrate biomonitoring (e.g., Peckarsky and Cook 1981, Chadwick et al. 1986, Vinyard and Watts 1992, Clements 1994, Clements et al. 2000, Clements et al. 2010).

The approach taken for the set of long-term collections summarized here has been to use bioassessment sampling at a reference site (Mountaineer) for contrast to a core group of exposed sites located below the Leviathan Mine AMD source, and above and below the confluence with Mountaineer Creek. Data on the chemical properties of sediments and water from each sample site have also been collected to aid interpretation of biological patterns but are not included in this report. Past trends have shown gradual improvements in biological conditions progressing upstream toward the mine site contamination source area (Herbst 2016). Previous reports have examined patterns of biological impairment over the greater Leviathan Mine watershed including samples from streams above the mine, on the receiving waters of the East Fork Carson River above and below inflow from Bryant Creek, and on reference streams adjacent to the watershed (Herbst; series of reports 1995-2015). As with previous monitoring, sampling was conducted in late spring (June 10-11) and near early fall (September 23-25), within the index periods established for this study (late May to mid-June, and late September).

Bioassessment sampling was conducted by collecting benthic invertebrates from riffle habitats in shallow stream sections within established survey reaches. Riffles are turbulent flows of water over rocky, shallow stream reaches and contain the greatest abundance and diversity of benthic stream fauna. Samples were taken by kicking and flushing organisms by hand from rocks for 20-30 seconds into a 250-micron mesh D-frame net held just downstream of the 25 x 25 cm sample area (width and depth of the net). Large wood or rock debris was washed and removed from the net and the sample procedure repeated at 2 more locations across each riffle transect. This composite sample of 3 collections was then swirled in a bucket, pouring off lighter suspended material to separate mineral from biological fractions (elutriation), the mineral fraction remaining in buckets was inspected in shallow white trays, remaining invertebrates collected, and the sample preserved in 95% ethanol. Such a collection contains benthic invertebrates in proportion to their relative abundance within the riffle sample areas. Five replicates of these composite kick-samples were taken at each site (moving upstream in randomly located riffle transects) as an estimate of spatial and sampling variability for statistical description and comparison. Field sampling was conducted by crews from the EPA Region 9 office, and AMEC Foster-Wheeler, trained and with field supervision by David Herbst, or Ned Black of the USEPA. The invertebrates collected were identified to the lowest practical taxonomic level (usually genus, species, or species group except oligochaetes and ostracods). Samples were sorted in the lab, organisms identified and counted, and data entered onto an Excel spreadsheet for analysis. Field samples were usually subsampled using a rotating-drum splitter, but some with low densities were counted in their entirety (counts per sample typically averaged between 250-500 organisms). Laboratory subsampling, processing, sorting and identifications were performed at the Sierra Nevada Aquatic Research Laboratory (SNARL), where subsample removal efficiency and cross-confirmation of all identifications were performed and recorded on sample log sheets and identification and count lists for each sample. Reference collections of all taxa have been established at SNARL to facilitate accurate identifications and for voucher archival. This provides a resource for comparing and verifying any taxa identified (preserved specimens and photos). For methods and QA/QC procedures, see: http://www.waterboards.ca.gov/lahontan/water_issues/projects/quality_assurance_project_plan/index.shtml.

Data were analyzed using descriptive statistics and graphical contrasts among sites and by season and time. The primary metrics used in interpreting community structure and biological integrity were based on measures of diversity, tolerance, density, and dominance. Mean taxa richness is a measure of overall taxonomic diversity for each site and should increase with heterogeneity of habitat, spatial complexity, and food resources. Mean EPT richness index is a measure of the diversity of generally sensitive insects belonging to the mayfly (Ephemeroptera), stonefly (Plecoptera) and caddisfly

(Trichoptera) orders and will increase in clean, cold, well-oxygenated waters exposed to minimal chemical pollution or habitat alteration (calculated as the sum number of taxa in these groups in each sample). The biotic index is a composite measure of overall community tolerance to pollution and will increase (over a scale of 0-10) as water and habitat quality are degraded (it is calculated as the product of relative abundance and tolerance value for each taxon, summed over all taxa). The percentage of midges, particularly certain tolerant taxa, often increases within the sample under degraded conditions of water and habitat quality. Dominance is a measure of the relative abundance of the most common taxon and a high proportion often indicates an imbalance or disturbance in food or habitat resources that permit one or a few species, such as midges, to dominate. Invertebrate density is often quite variable and less reliable as an indicator, but when pollution is severe, density of even tolerant taxa can be reduced as stream conditions become unsuitable. Density also may provide a measure of natural seasonal population recruitment during the summer growth period.

In addition to the use of the metrics above, invertebrate communities were also described in terms of food web structure summarized by trophic group (algae grazers, fine and coarse organic particle feeders, and predators). As an exploratory analysis of other factors contributing to variation in invertebrate species composition, differences between seasons at the Mountaineer reference sites were quantified using non-metric multidimensional scaling (NMS) in PC-Ord (McCune and Mefford 1999), with Sorensen distance as the measure of community dissimilarity.

Results and Discussion

Quality Assurance Memorandum

From the 2015 sampling covering 15 surveys (8 in June & 7 in September), nearly 27,000 individual organisms were counted and identified from 75 samples, comprised of 181 taxa (365 total over history of project). Removal efficiency of invertebrate specimens from subsamples exceeded 95% in all cases, and identifications, counts and certainty of taxonomic designations were checked and completed for all taxa (averaging over 350, with just 4 samples having <100 organisms counted). Invertebrate collection vouchers were archived for all subsamples identified and counted as well as the field sample remnant. To minimize underestimates of diversity by subsampling, large and rare taxa were removed from each remnant sample and added to the data list as single counts if they did not occur in the subsample counted. Densities were determined based on the subsample fraction counted for each sample and the area sampled. Data were compiled in an Excel spreadsheet of taxa found for all years of the project.

Annual Trends by Site

The Leviathan watershed map of sites is shown in Figure 1, and hydrographs for the USGS gauges on the East Carson River and Bryant Creeks are shown in Figures 2 and 3, respectively. Summary of annual trends in primary indicator metrics are given in Figures 4-9, and reference stream standards given in Figures 10-12. Note that for clarity of presentation only the means of the metrics (for the 5 sample replicates in each case) are shown in all the trend graphs, and each sample period is in sequence (some years without seasonal samples). The coefficients of variation of the principle metrics within sites for each date range from 5-10% for the biotic index, 10-20% for richness metrics, 25-50% for density and 15-40% for dominance. In previous surveys over this set of sites the most prominent pattern was of poor biological performance measures at the sites closest to the mine source area (Leviathan below mine, Leviathan above Mountaineer, and Aspen below mine). Over time there has been progressive recovery, attaining conditions within the range of Mountaineer Creek and other reference sites, and this appears to be related to control of AMD discharge.

<u>Mountaineer Creek Reference</u>. For most metrics, the trends observed in Mountaineer Creek have both been more stable and indicative of high quality biological conditions compared to trends observed in Leviathan Creek and Bryant Creek over the record of surveys. Metric values for 2015 were within the previously observed range in all cases (Figures 4-9). The pattern of lower spring densities than in the preceding and following fall observed in most average to wet years of runoff was not observed this year, as in other drought years with low spring runoff and higher densities found in part because habitat area contracted and flows did not flush organisms and organic matter downstream (seen also in 2012-14). The overall stability of metrics at this site attest to the continued

quality of Mountaineer in representing the natural reference state (coefficient of variation for biotic index and diversity measures comparable to the best metric performance for regional reference sites in the range of 10-15% (Herbst and Silldorff 2006). The consistent abundance and variety of benthic invertebrates at Mountaineer Creek suggest the absence of AMD exposure promotes more growth and productivity of a stable and balanced community. The food web at Mountaineer and other reference sites reflects this balance in composition, with higher densities of algae grazers and large predators than at AMD-exposed sites (Figure 13). Both these groups forage on, and cling to or between rock surfaces, so deposits of yellow-boy and inadequate access to quality food sources may limit these groups in particular at the AMD-exposed sites, with downstream Bryant Creek sites showing structure and density of collector gatherers and filterers (CG and CF), and shredders (SH), indistinguishable from reference streams.

Flows and runoff timing may have important effects on stream invertebrates. Although years 2000-2004 were below average water years (Figure 2), and had low winter-spring cumulative flow during this period in 2001 (Figure 3), coinciding with a drop in EPT taxa in Mountaineer Creek (Figure 6). More recent drought has shown no such declines in diversity. Low antecedent flows in winter-spring did not result in declines in June EPT in 2012-2015 outside the normal range. Although species diversity may not respond to altered hydrology, the species composition at Mountaineer was found to change between seasons, with the dissimilarities most pronounced during extreme flow conditions in 2013-2015 drought years, and the wet year of 2006 (Figure 14). Total and EPT diversity have always been higher in Mountaineer than at any AMD-affected site until recovery began to occur on Bryant Creek sites after about 2007. With little flow and reduced habitat area during drought, high spring densities contrast with the high flow scour of spring 2011 which appeared to produce lower invertebrate densities across all sites including Mountaineer (Figure 8), as observed in most other years of high spring runoff such as 1997 (record winter flood), 1999, 2006 and 2010 (Figure 2). With the high flows there have been higher values of biotic index and taxa dominance (Figures 7 and 9), attributable to a preponderance of midges at Mountaineer. Midges also tend to be more common in spring than fall at most other sites. Seasonal increases in density from spring to fall at Mountaineer appear to recur with regularity in years of normal runoff, suggesting that natural population demographics follow this pattern, as recruitment, growth and development of many populations occur over this time period. Spring runoff scour under high flows may accentuate this difference, while drought seems to reverse the pattern. Most years with spring densities higher than fall were preceded by drought or during low flow conditions. Several of the recovering AMD-exposed sites have also begun exhibiting seasonal spring to fall increases, indicating return of typical population cycles in years of average runoff.

Framing the Nearby Reference Stream Condition of the East Carson Watershed:

In order to evaluate metrics of diversity and tolerance at other reference sites that have been sampled over the lengthy monitoring period of this project, data were compiled from the 7 other streams that have been sampled over one or more seasonal cycles (Figures 10, 11, 12). The range of values shown can be used to develop stronger inferences of impact from AMD than Mountaineer Creek data alone. These data plots show that site means for a given date can be considered in an impaired range if having a biotic index exceeding 4.5, mean total taxa richness less than 30, or mean EPT richness less than 12 (mostly outside the range exhibited in these reference sites). More stringent standards using reference data would use values below the 10^{th} percentile of these and Mountaineer Creek data combined to specify limits on acceptable biological conditions as practiced in California bioassessment (Mazor et al. 2016).

Aspen Creek Below the Mine (Abm). Although gradual recovery at Aspen Creek below the mine, first noted in fall of 1999 as an improved (e.g., decreased) biotic index (Figure 7), had continued with the accrual of both mean number of total taxa diversity through 2004 (Figures 4, 5, 6), total richness measures declined from 2005 to 2006, rebounded starting in 2007 and have been variable through the drought, but EPT diversity has remained in the sub-reference range. The biotic index has also remained mostly above 4.5, in a range showing that taxa are of the type that are more tolerant of poor water quality than found in the reference condition (Figure 7). Initial recovery at Aspen Creek involved colonization by opportunistic taxa including the mayfly *Baetis* and the black fly *Simulium*, followed by the Nemourid stoneflies *Malenka* and *Zapada*. From low levels of abundance, the density of invertebrates had gradually increased at this site, declined again in 2009-2010, then increased in Fall 2011-2012. and stabilized 2013-2015 but still at levels well below reference (Figure 8). Instability in diversity, low density, and poor EPT numbers suggests that even though a mixed community is becoming established at this site, it has not yet recovered to reference condition. The fluctuations of diversity, tolerance, density and dominance may be at least partly attributable to repeated livestock trampling of this small stream at the sampling locality in 2004-2006, and to high flow in 2011. Collapsing banks, crushed and muddy ground cover, and erosion that were observed during 2004-2006 had not been noted in previous sampling and were stopped by 2008. During this time there has been continual upstream treatment of stream flow through the Aspen Seep bioreactor, but other small seep AMD sources may exist between the bioreactor, and the sample site much further downstream. Despite improvements in water quality, metrics through 2015 on Aspen Creek were mixed, some meeting and others not meeting reference stream conditions.

<u>Leviathan Creek below the mine and above Mountaineer Creek (Lbm & LaM)</u>. In 2003, the Leviathan Creek below mine site, closest to the mine, had shown some early signs of recovery – increased taxa diversity, EPT numbers, reduced biotic index values, and lower levels of dominance by tolerant chironomids, though total density still remained low (Figures 4-9). In 2005-2006 these improvements were reversed, with losses in the diversity and density, and rising biotic index and dominance. Under what appeared to be a more effective and prolonged control of AMD discharges, the 2007-2008 levels of richness again showed an improving trend. In 2009 this site had flows only in spring and dried by fall, and this occurred again in 2012-13-14. Although conditions improved in fall 2011, with a period of good flows in that year, metrics have since fallen far short of reference condition, and high biotic index and greater dominance of pollution-tolerant midges such as *Eukiefferiella claripennis* show this site continues to be inhabited mainly by taxa capable of living in poor water quality. Densities have remained very low, and while total and EPT diversity achieved record high values in Fall 2011, these declined again when flows were low 2012 through 2015. Drying of this site remains an additional stress, and site 4L substitute (map Fig 1) also showed poor metrics (mean EPT = 3.0 in spring, 3.6 in fall; data from this site substituted Lbm Fall 2014 and Fall 2015).

Further downstream, Leviathan at Mountaineer (LaM, above Mountaineer confluence), had also exhibited similar patterns of progressive recovery into 2004, evident in stabilization of the biotic index (as was noted in the initial recovery phase of Aspen Creek) and continued increase in diversity and density. The amount of yellow-boy deposition at this site had also appeared to be declining. The 2005 and 2006 surveys showed that recovery here too had been reversed – evident in losses in diversity and density and increase in biotic index in 2005 after slight gains in 2004. Low levels of density of benthic invertebrates such as those observed at these Leviathan Creek sites shows how severely AMD can depress biological activity and biomass production. Low density remains a feature of Leviathan Creek. Just above the inflow of Mountaineer Creek, this lower Leviathan Creek site showed that without dilution by uncontaminated flows, biological integrity had deteriorated during 2006. While conditions have been instable since 2007, as of Fall 2011 and continuing through 2013, the trends have shown the highest richness scores ever observed at this site (Figures 4-6). The flushing of contaminated sediments and dilution of dissolved metals during high runoff of 2011 may have promoted the onset of this recovery, continued through drought years of 2012-2015. In Fall 2014 this site suffered losses of diversity and density, but improved again in 2015. This site best reflects fall recovery after summer treatments, followed by relapse in spring after an absence of overwinter capture of AMD (Figure 14).

Bryant Creek. At sites below the mixing zone with clean flows from Mountaineer Creek, biological impairment has usually been less apparent than at Leviathan above Mountaineer and the sites immediately below the mine. The Bryant below confluence sample station and Bryant middle station (also known as the Stateline site) appear to be the locations where the most extensive recovery has occurred and persisted in 2004-05 even while the Delta Seep releases were untreated. In 2006, the Bryant sites lost diversity (though maintained EPT), and had variable levels of density and dominance. At the same time, loss of sensitive taxa and/or gains in tolerant organisms were occurring below the confluence (increased biotic index, Figure 7), but not at the Stateline site downstream. While streambed substrates in these areas still showed traces and deposits of yellow-boy iron oxides, these sites were once densely covered by this precipitate when sampling began in 1995 and 1997. In the early stages of recolonization, these sites contained elevated numbers of some pollution-indicating taxa such as certain midges (e.g. *Eukiefferiella claripennis* grp., *Corynoneura*), empidids (*Chelifera /Neoplasta*), and mites (*Sperchon*), but have accumulated more total diversity and EPT taxa with time. The variable early trends associated with these locations may be indicative of instable habitats in transitional phases of recovery, but absence of severe change in biological condition suggest sustained health and further recovery are ongoing on upper Bryant Creek. As of 2009-15, the Bryant Creek sites appear to be benefiting from reduced AMD discharge as they are consistently within the range of the reference conditions at Mountaineer Creek and the external reference sites. These sites also have been exhibiting the natural spring-fall cycle of density increase since 2007 (except during recent drought as noted above for Mountaineer), further providing evidence that these sites are in recovery. Re-sampling of Bryant Creek above Doud spring was conducted in 2010 through 2013 and metrics at this site all indicate improvement to near-reference range, as was seen in 2000 after having been in an impaired range prior to that. Sampling at this site has been discontinued as of 2014. In 2015, Bryant below confluence and at Stateline showed indicators maintaining reference range.

<u>General Patterns</u>. Annual and seasonal trends for selected sites over the monitoring period 1997 or 1998 to 2015 is used in most of the data presented. Although sampling began in spring of 1995, the method used then involved collection from only one sample area for each of 3 replicates (resulting in low counts), while all other samples from 1997 forward had sufficient counts or collected three combined samples for each of 5 replicates. The 1995 data will therefore underestimate measures of diversity and community composition. The mean taxa richness (Figure 5) shows that this measure of total diversity is typically in the range of 35 to 50 taxa at the Mountaineer reference site, and mostly less than about 30 at the Leviathan and Aspen AMD-exposed sites, but mostly within reference range on Bryant Creek by 2003. Improving trends were apparent in 2003-2004 at all sites and again by 2008 after degrading some in 2005-06, and include some early signs of recovery at Leviathan Creek nearest the mine. As conditions have improved in AMD-impaired streams, the community shifts from one of low-diversity,

inhabited typically by a few species of very stress-tolerant organisms, to a transitional community of instable composition, dominated by "weedy" species (opportunistic colonizers such as the mayfly *Baetis*, and the black fly *Simulium*) that are often tolerant of metal contamination, and a mix of more sensitive organisms. As improved water and habitat quality conditions persist, this instable phase is expected to be replaced by a more abundant, diverse and stable community of more equally-represented taxa, with varied food and habitat uses, and regular seasonal patterns of population demography. Evidence of such patterns in community structure are present in unpolluted streams and during more complete effluent treatment periods, and should become more clear and predictable with continued trend monitoring during the ongoing remediation of AMD.

Stages in progressive biological degradation or recovery related to AMD contamination may be discerned from changes in certain indicator organisms. About one-third of the total taxonomic diversity is found within one family - the Chironomidae or midges. Within this group are some of the best indicators or signal taxa for discerning water quality impact. Imbalance in community structure may first become apparent at moderately polluted sites (or those in initial stages of recovery) where *Baetis* alone may come to dominate >50% of all taxa. As severity of AMD exposure increases, *Baetis* abundance decreases while the relative abundance of midges often increases. With further pollution the midge community itself comes to be dominated by *Corynoneura* and *Eukiefferiella claripennis* sp. group. Other taxa that appear in smaller numbers but are most prevalent at polluted sites include the empidid genera *Chelifera/Neoplasta*, the midges *Pseudorthocladius*, *Pseudosmittia*, the crane fly *Molophilus*, and the biting midge *Monohelea*. *E. claripennis* dominates where AMD pollution is chronic, and is present only in low numbers at unimpaired sites. It is not clear if these insects possess a general tolerance of physiological stress or a specific capability to resist low pH and toxic metals. The *E. claripennis* group is a known indicator of degraded water quality conditions (Bode 1983), and has been abundant in Aspen and Leviathan Creeks below the mine in spring, becoming much less numerous in fall, possibly related to population phenology, or to deteriorating water quality beyond the tolerance even of this species. Recovering communities are first recolonized by opportunistic taxa with rapid growth (*Baetis* and *Simulium*), and by a more diverse group of moderately sensitive taxa that are common and widely distributed (e.g. *Malenka*, *Zapada*, *Ceratopsyche*, *Pagastia*, *Optioservus*). Dominance by these groups is then reduced as more sensitive EPT taxa can become established with further easing of AMD stress. Examples of how combined metrics and overall community similarity can vary between sites and over time have been shown in other long-term monitoring studies (Clements et al. 2010).

The decreased abundance and diversity of benthic macroinvertebrates in AMD-affected streams is a well-documented phenomenon (reviewed by Hogsden and Harding 2012), but there are few examples of how biological recovery proceeds over time with treatment of effluent, and with natural seasonal and inter-annual environmental variation. In this regard the Leviathan data set provides an important case history in establishing the success of AMD remediation activities. The use of biomonitoring as an indicator of ecological toxicity and mining-related pollution impacts and improvements has been substantiated through studies that show close correlation of bioassessment metrics with the standard bioassays using specific test organisms, and with dissolved metal contaminant concentrations (Schmidt et al. 2002, Griffith et al. 2004). Field studies on streams in the mining district of the upper Arkansas River in Colorado showed that within two years following water treatment that removed metals from contaminated inflows, EPT taxa increased and bioassessment metrics achieved upstream reference condition (Nelson and Roline 1996). Similar treatments on the Clark Fork in Montana required much longer periods for aquatic invertebrate recovery to occur (Chadwick et al. 1986), but were complicated by flows redistributing metal-contaminated sediments (Hornberger et al. 2009). Bioassessment monitoring of the Leviathan Creek watershed has also shown mixed results, with recovery occurring during periods of effluent control to the stream, and relapse to degraded conditions when AMD pollution has not been abated (2005-06), or when unrelated disturbances such as livestock grazing incursions have occurred on Aspen Creek (prior to 2006). The results from 2011-15 during a year of high runoff followed by drought years indicate that even though densities may be reduced during scouring spring flows, this benefits enhanced species diversity by early fall in streams previously impaired by AMD exposure, and continues into drought, but low flows may compromise recovery in some cases.

The algae and organic matter food resources of benthic invertebrates may become reduced in streams exposed to AMD. Growth of most algae on stream bed surfaces is severely decreased under lower pH, elevated metal concentrations and when metal hydroxides such as yellow boy coat and cover substrata (Niyogi et al. 1999, Verb and Vis 2001). Microbial decomposition of leaf litter and wood that fall into streams is an integral trophic resource in forested watersheds, and the bacteria and fungi that mediate this process may be impaired by AMD (Niyogi et al. 2002, Schlief 2004). These results show that AMD may alter ecosystem processes of primary production and decomposition, changing food resource availability and distribution, forcing food webs into simpler and less productive pathways. These kinds of changes in organization of Leviathan stream communities can be examined in terms of the functional feeding group structure between and among sites over time. Such a trophic analysis may contribute to a more complete understanding of AMD impact and recovery on stream ecosystems.

The mechanisms of alteration to benthic invertebrate communities by AMD are likely related to a mixture of factors. Direct mortality caused by high concentrations of toxic metals and low pH, along with exclusion from rock surfaces and interstices by yellow-boy deposits may be most common where pollution is severe. Mild acidification from neutral pH of 7 to 5.9 was shown in experimental treatments of a stream to increase drift of mayflies, midges and caddisflies, so even without causing direct mortality, modest acidification can change the composition of stream benthos (Bernard et al. 1990).

AMD poses multiple stresses on benthic invertebrate communities. Chemical stressors include a mix of toxic dissolved metals (e.g., As, Ni, Al), and deposits of iron oxide yellow-boy. Given the physical effect of chemical precipitates that can cover surfaces, this may prevent inhabitation of substrata. It may be possible to account for the presence and extent of these coatings in the iron oxide content given in sediment quality samples (such as those collected at Leviathan by N.Black of USEPA). These data could be used to help separate the effects of this precipitate coating from the effects of sediment-bound metals, aqueous metals, and pH using a multiple regression analysis. Long-term assessment using a metal-specific sensitivity among BMIs may provide an additional index for establishing the severity of ecological impairment from AMD. Such an index developed for BMIs in New Zealand streams affected by AMD showed strong correlation with diversity metrics such as EPT richness, and improves the reliability of biomonitoring data in showing stream ecosystem recovery that can be directly linked to elimination of stress from AMD and metals toxicity (Gray and Harding 2012). The combined effects of metals can also be assessed through use of CCUs (cumulative criterion units) which express instream concentration relative to EPA toxicity criteria for mixed metals (Clements et al. 2000). Even though metals may be reduced by AMD treatments, high conductivity remains in Leviathan Creek, with elevated levels of sulfate posing potential ionic and osmotic imbalances for some species. Bioassays of treated water, with and without sulfate removal by precipitation with barium, would be useful for assessing treated water effluent effects.

Long-term assessment of the biological integrity of streams in the Leviathan Mine watershed will require continuation of a monitoring program to ensure data are available to inform adaptive management objectives. Sampling in both spring and fall produces information on seasonal and demographic shifts, revealing natural patterns in community and population ecology as well as problems arising from incomplete control of mine pollutants at different times. Monitoring at Aspen and Leviathan below the mine will provide a measure of the most difficult conditions for recovery nearest the source areas of contamination, while survey of Leviathan and Bryant above and below Mountaineer provides ongoing feedback on the success of treatment activities in ultimately restoring stability in ecological integrity to reference quality. Sampling at Mountaineer and other control stations, some external to the Leviathan watershed, will continue to be useful in framing the target range for attaining the desired condition of unimpaired community composition. Sustained recovery at the above Doud and Stateline sites on Bryant Creek suggest monitoring could be done less frequently at these locations because continued sampling has consistently demonstrated metric values within or near the reference range.

<u>Expectations for the Influence of Drought and Reduced Flow on Streams Communities:</u>

Streamflow alterations have been shown to degrade stream biodiversity. Bioassessments of streams across the country have shown that among mixed chemical and physical variables, diminished flow magnitudes were the primary predictors for loss of biological integrity among fish and macroinvertebrate communities (Carlisle et al 2010). Trait states shifted to pool-dwelling taxa tolerant of sediments and slow currents.

Flow reductions from 1980-2007 (due to diversions, impoundments) were especially severe in the Sierra Nevada, with low gradient streams exposed to low flows showing the most impaired biological integrity. The syndrome of changes include reduced extent of stream area, altered habitat types and resources available, disconnection of habitat and increased importance of protected flow refugia. Without flow to transport materials, fine sediments are deposited, and algae along with detritus and leaf litter accumulate, and nutrients can become concentrated. As the area becomes confined, there are initial increases in abundance of invertebrates, loss of the diverse rheophilic or current-loving species and increase in tolerant forms (Rolls et al. 2012). Progression of drying leads to mortality and reduced numbers and declining diversity of sensitive taxa while tolerant forms increase. Some of this comes from direct impacts of habitat loss, but may also be attributed to initial increase in density-related interspecific competition and predation rates as the volume of habitat concentrates benthic fauna.

Seasonal droughts occur regularly in arid region streams while supra-seasonal drought refers to extended and unpredictable periods of drying (as began in 2012) that produce intensifying stress as marginal stream areas dry, shallows become warmer, and as habitat is compressed the flows typical of lotic stream environments become increasingly lentic or ponded in character (Lake 2003) and may even develop intermittent flow patterns. In the Leviathan watershed streams, an increase in invertebrate densities have been observed through 2015,and while total and EPT diversity have not shown signs of loss, community structure has been altered between spring and fall seasons (Figure 14), though less so than seasonal variation at AMD-exposed sites.

Summary of Trends and Conclusions:

Bioassessment monitoring in the Leviathan Mine watershed has shown varied responses in biological integrity on sites exposed to AMD from 1997 through 2015. After a modicum of initial improvement in benthic invertebrate indicators, surveys performed in spring and fall of 2005 and 2006 showed that the communities of Leviathan

Creek, Aspen Creek, and Bryant Creek had lost richness and density, and were comprised of pollution-tolerant types of taxa. Slowing and reversal of recovery corresponded to an uninterrupted AMD effluent discharge of Delta Seep to Leviathan Creek during 2004-06. In contrast, more recent data through 2015 attest to improved conditions across Bryant Creek sites, approaching reference stream metrics, and mixed responses on Leviathan and Aspen Creeks. The instability of community structure and tolerance measures over time at the sites closest to AMD-influence indicates these locales are still in a state of shifting composition and functionality as exposure to chemical pollution fluctuates. In both years of high runoff and drought in 2011, and 2012-2015, high levels of benthic invertebrate biodiversity have been supported and sustained at references and downstream AMD-affected sites but upstream sites nearest the mine continue to show impacts.

The following recommendations are based on monitoring data to date:

1. In order to interpret how different remediation activities are related to changes in the stream communities of the Leviathan Mine drainage, the biological response patterns should be coupled to a chronology of the timing, locations, and types of operations that have affected the volume and quality of treated flow. This discharge information, along with water chemistry data, will permit evaluation of the effectiveness of individual and cumulative treatments, and correlation of chemical improvements in water and sediment with ecological recovery.
2. Further analysis of the complete bioassessment dataset to include (1) community ordination of taxonomic similarity (such as non-metric multidimensional scaling) to graphically distinguish over time how changes in the invertebrate fauna of AMD-exposed sites compare to the fauna of local and external control sites and are related to metal contaminants of water and sediments, (2) an indicator species analysis using ordination to associate particular taxa with tolerance or sensitivity to particular pollutants or to a combined metals index (e.g., CCU), and (3) a comparison of the food web dynamics of the stream through partitioning of the changing functional feeding group composition of the invertebrate communities with time at different sites. [note: these analyses are being incorporated in a draft peer-reviewed publication, with some included in this report]

3. Continue seasonal sampling at the other established stations and periodically include external reference streams to frame the range of expected natural variation of the benthic macroinvertebrates of intact stream ecosystems. This is especially important recognizing the seasonal patterns of recovery and relapse shown in the data, and the importance of flow regime influence on toxic metals loading.
4. Incorporate bioassay studies of the effects of metals chemistry over the network of sites, on the decomposition of leaf litter and the microbial diversity of substrate utilization under differing conditions. This is relevant to the food quality available to invertebrates in streams where much of the ecosystem energy flow comes from leaves and wood that fall into these forested streams and become an important food source because of microbial growth and conditioning.

Acknowledgments

Field work and discussion of data has been done in collaboration with Ned Black, of USEPA, who has been an integral part of this project in collecting concurrent water and sediment chemistry samples, and providing quality control advice to others involved in data collection at Leviathan. Gratitude to Kevin Mayer and current project oversight by Lynda Deschambault (USEPA) who have consistently advocated for the utility of this data, as have representatives of Atlantic Richfield (Anthony Brown) and the Lahontan Regional Water Quality Control Board (Doug Carey and Hannah Schembri). Tom Suk (formerly of the Lahontan Board) was instrumental to initiating and enabling early days of the monitoring program, and ensuring rigorous bioassessment science is applied to management. Greg Reller has provided helpful feedback in report editing. Daniel McMindes and Cory Kroger of the US Army Corps of Engineers have been key in managing the contract process and facilitating working relationships. Peter Husby of EPA Region 9 has conducted and coordinated field sampling, and Mark Antwine has contributed assistance with past invertebrate identifications. Gene Mancini has also provided regular aid and quality control to the field work effort, and in report review. Thanks are due to this team, and all who have supported this work. Past support has been provided by the USFS, ARCO, LRWQCB, the USEPA, the US Army Corps of Engineers, and Superfund. Contracting through Weston Solutions, AMEC Foster Wheeler and current support from ARCO provide important continuity to the monitoring of effective remediation by a dedicated and collaborative partnership.

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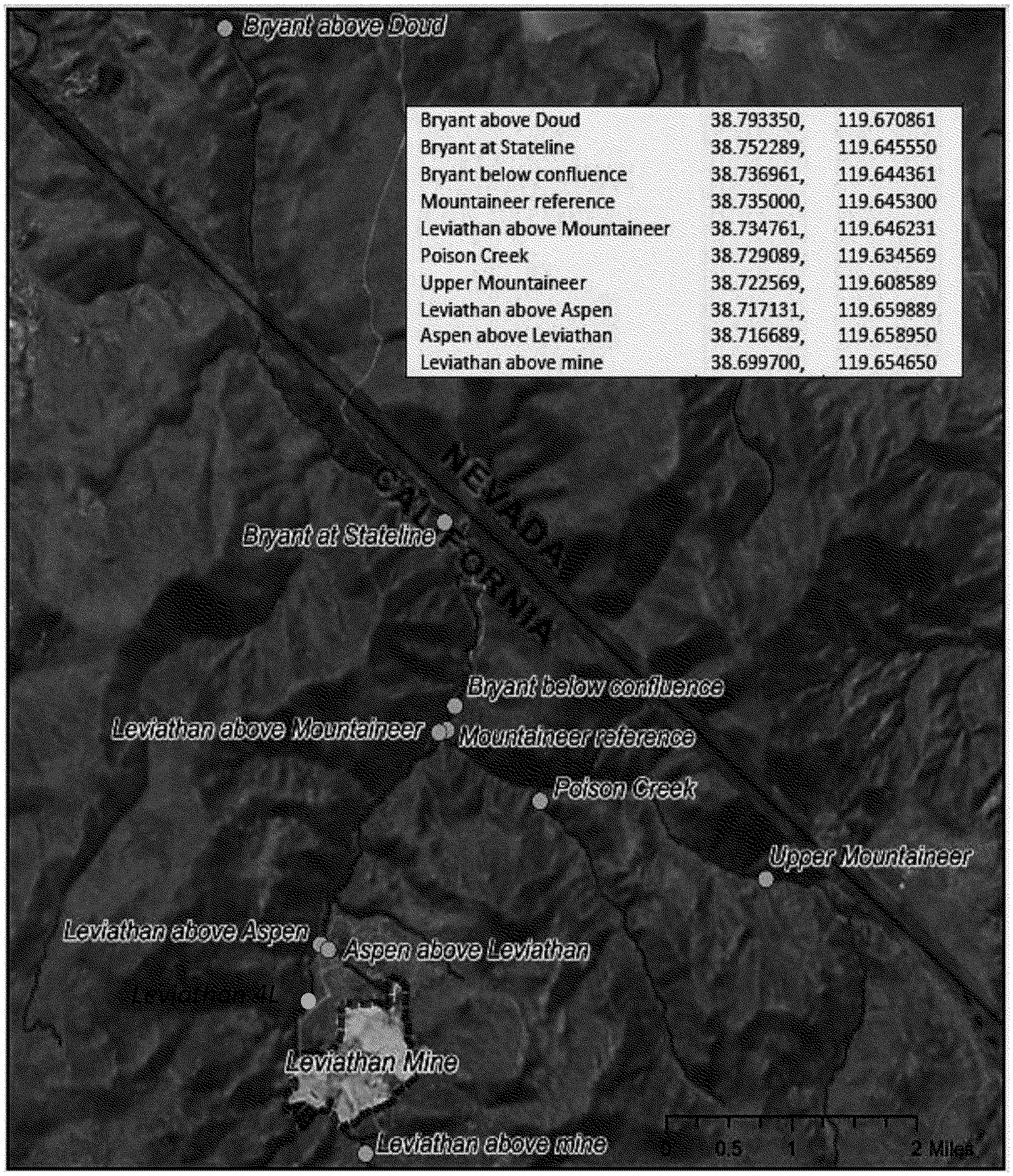

Figure 1. Locations of key sample sites surveyed for aquatic invertebrate biomonitoring of the Leviathan Mine watershed, and table of coordinates.

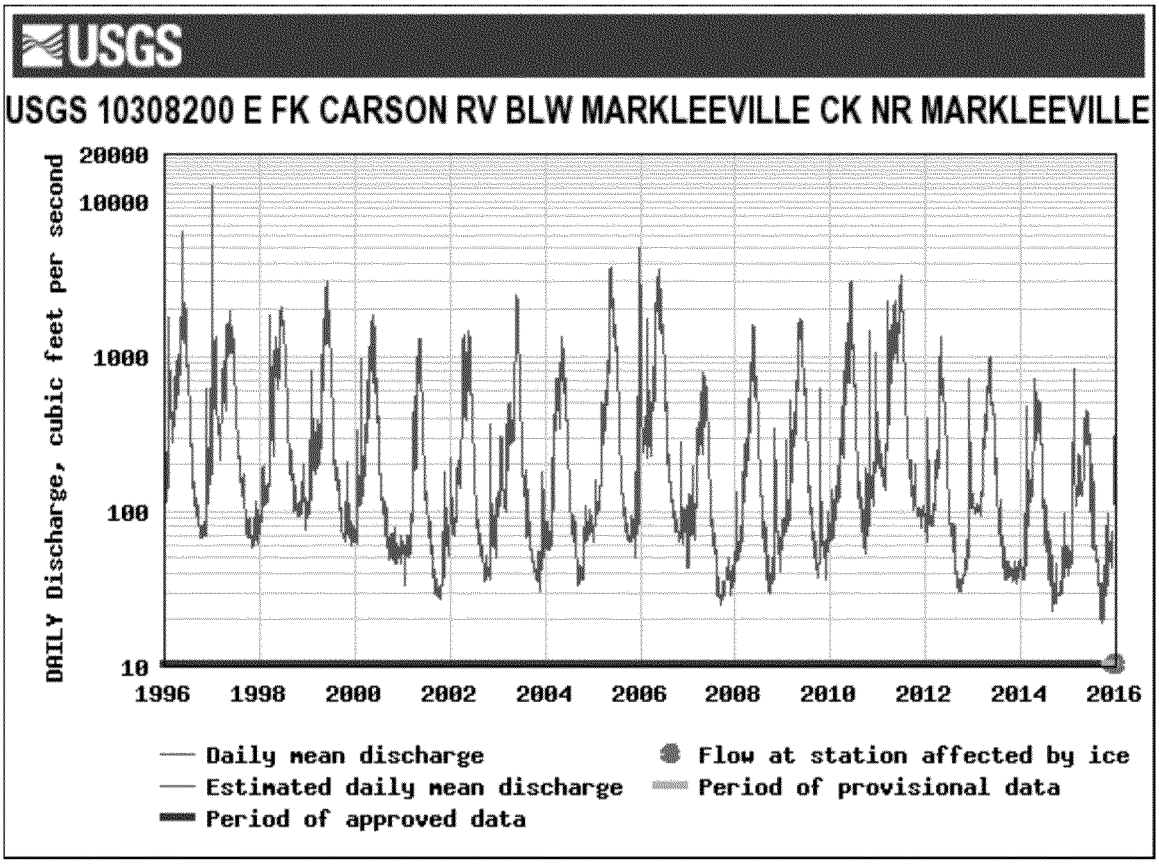

Figure 2. USGS hydrograph for E Fork Carson River (downriver of Bryant) 1996-2016.

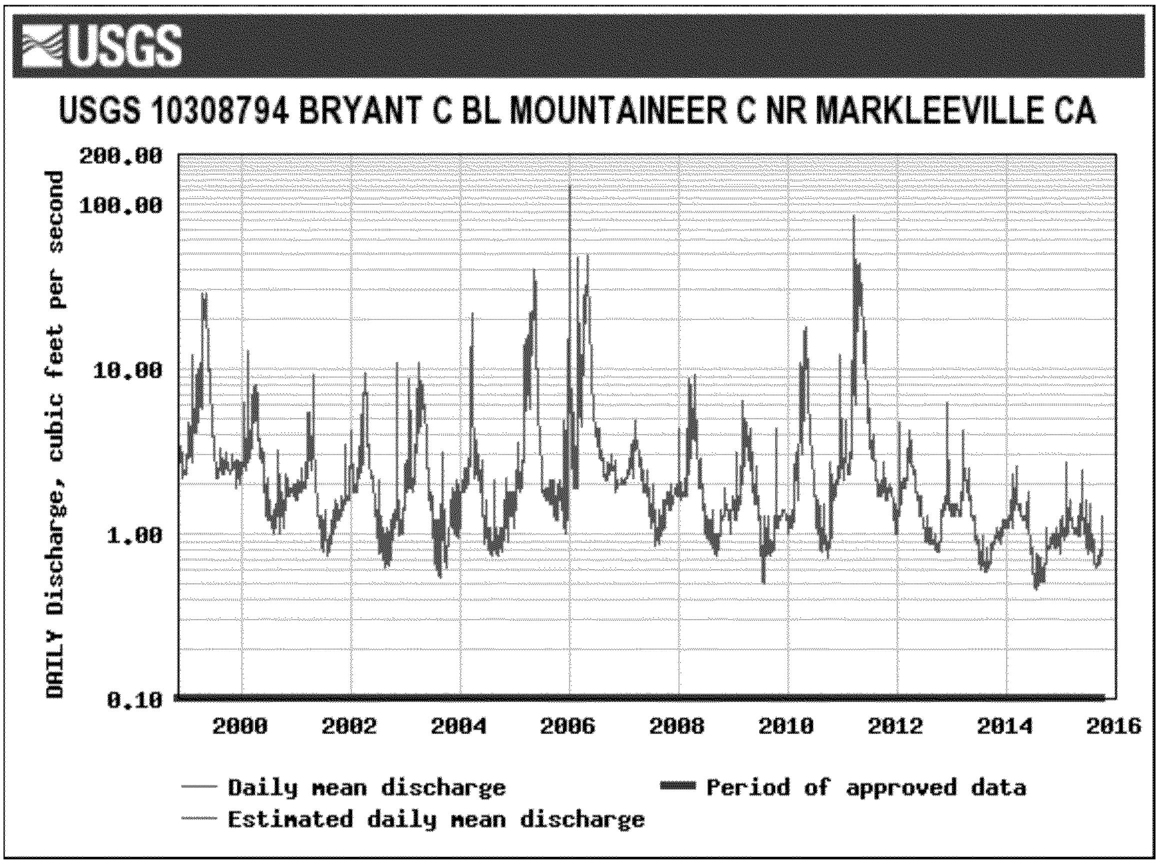

Figure 3. USGS hydrograph for Bryant below Mountaineer Creek for 1998-2015.

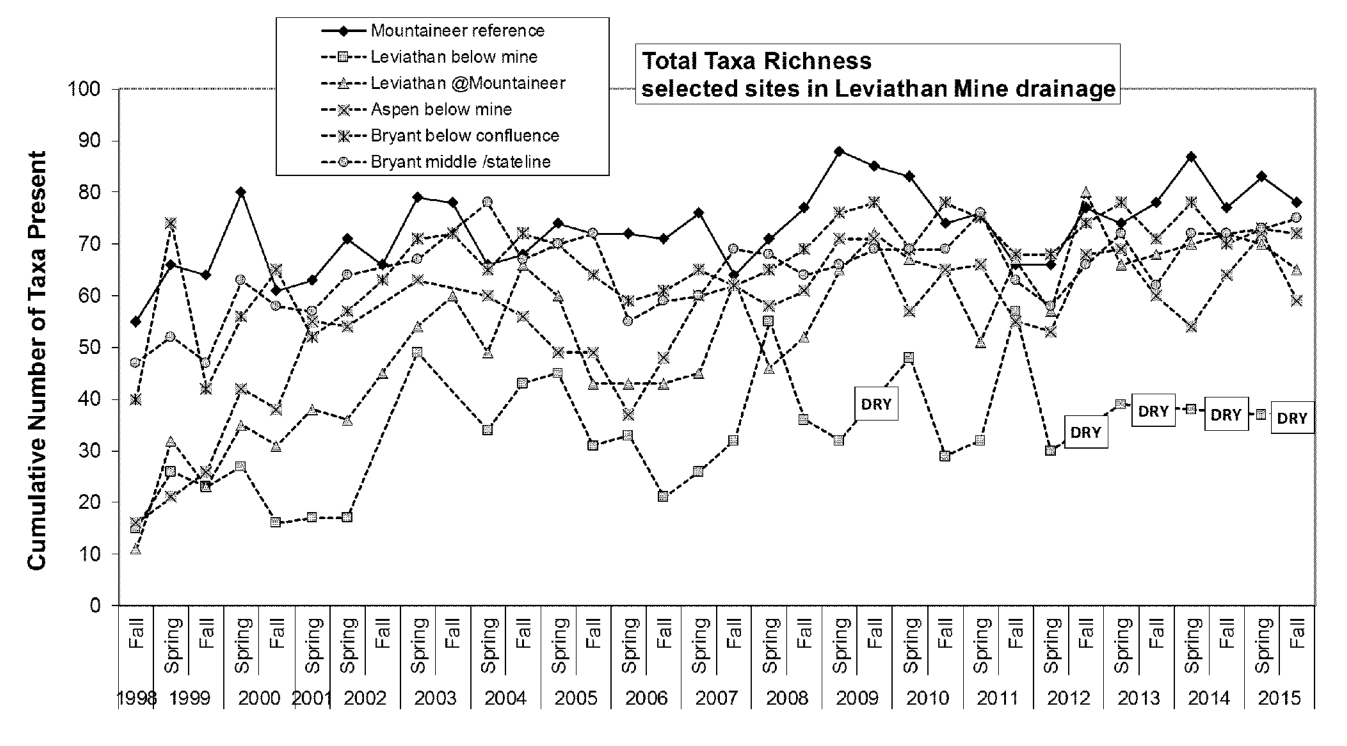

Figure 4. Richness expressed as the combined number of total taxa present from 5 samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites.

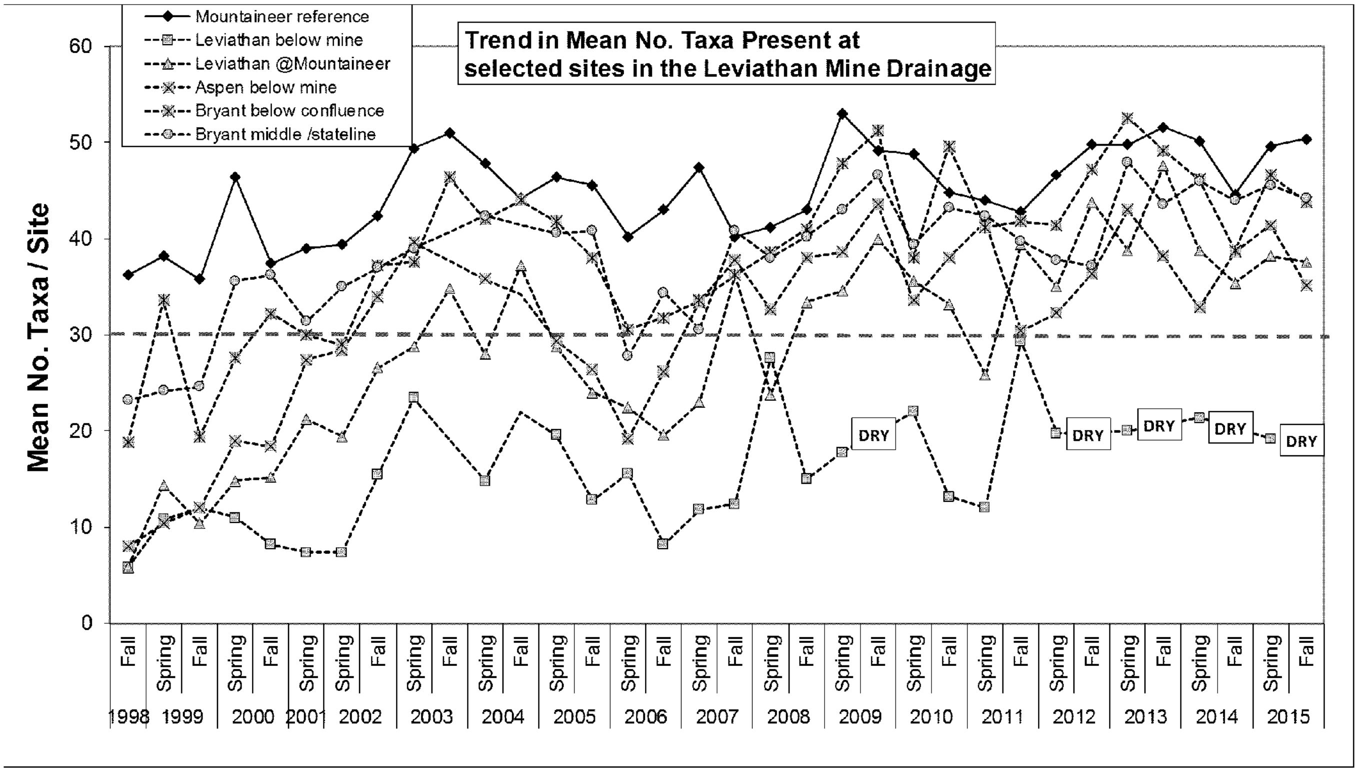

Figure 5. Richness expressed as mean number of taxa present in the 5 replicate samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites. Red line displays the 95% confidence limit derived from other local reference site collections (below line fails to meet reference quality; see Figure 11).

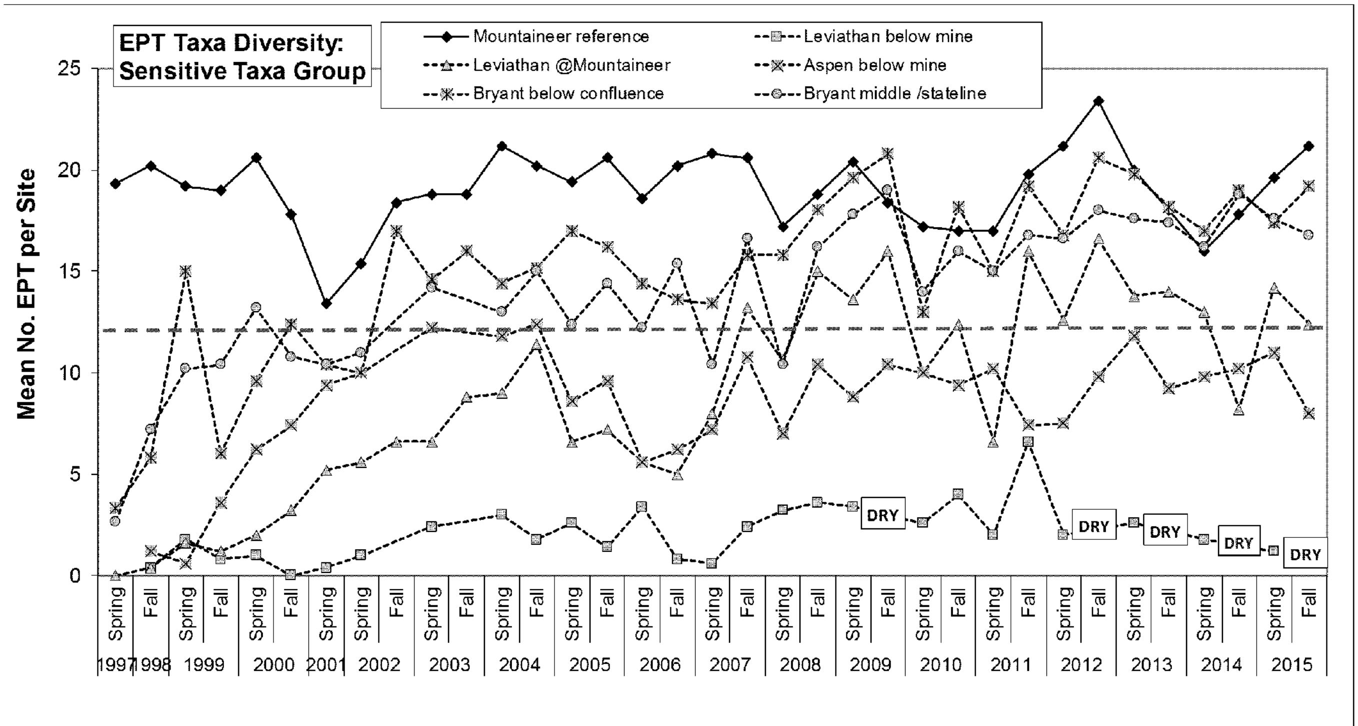

Figure 6. Richness expressed as mean number of EPT taxa present in the 5 replicate samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites. Red line shows the 95% confidence limit derived from other local reference site collections (below line fails to meet reference quality; see Figure 12).

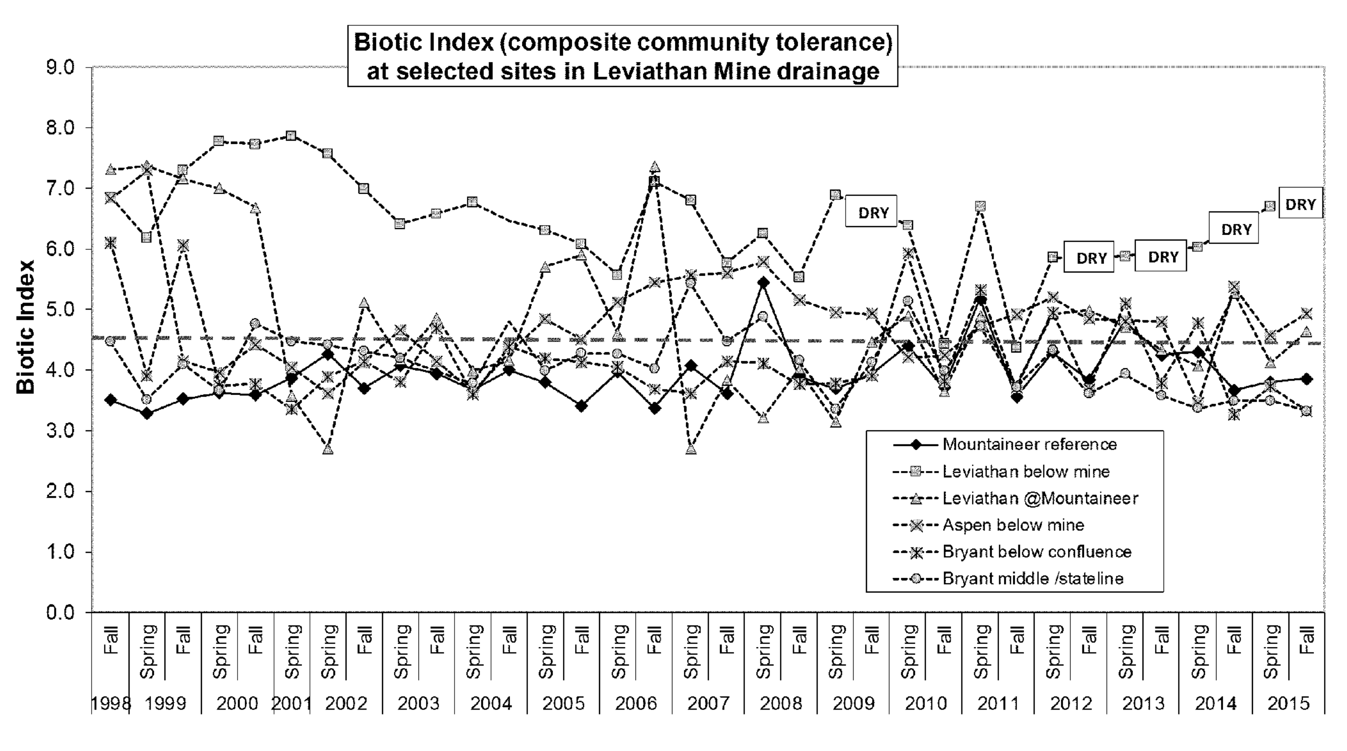

Figure 7. Biotic Index as the mean of 5 replicate samples at each site over time (season and year) for selected sites in the LeviathanMine watershed. Solid line for reference site, dashed lines for AMD-exposed sites. Red line shows the 95% confidence limit derived from other local reference site collections (above line fails to meet reference quality; see Figure 10).

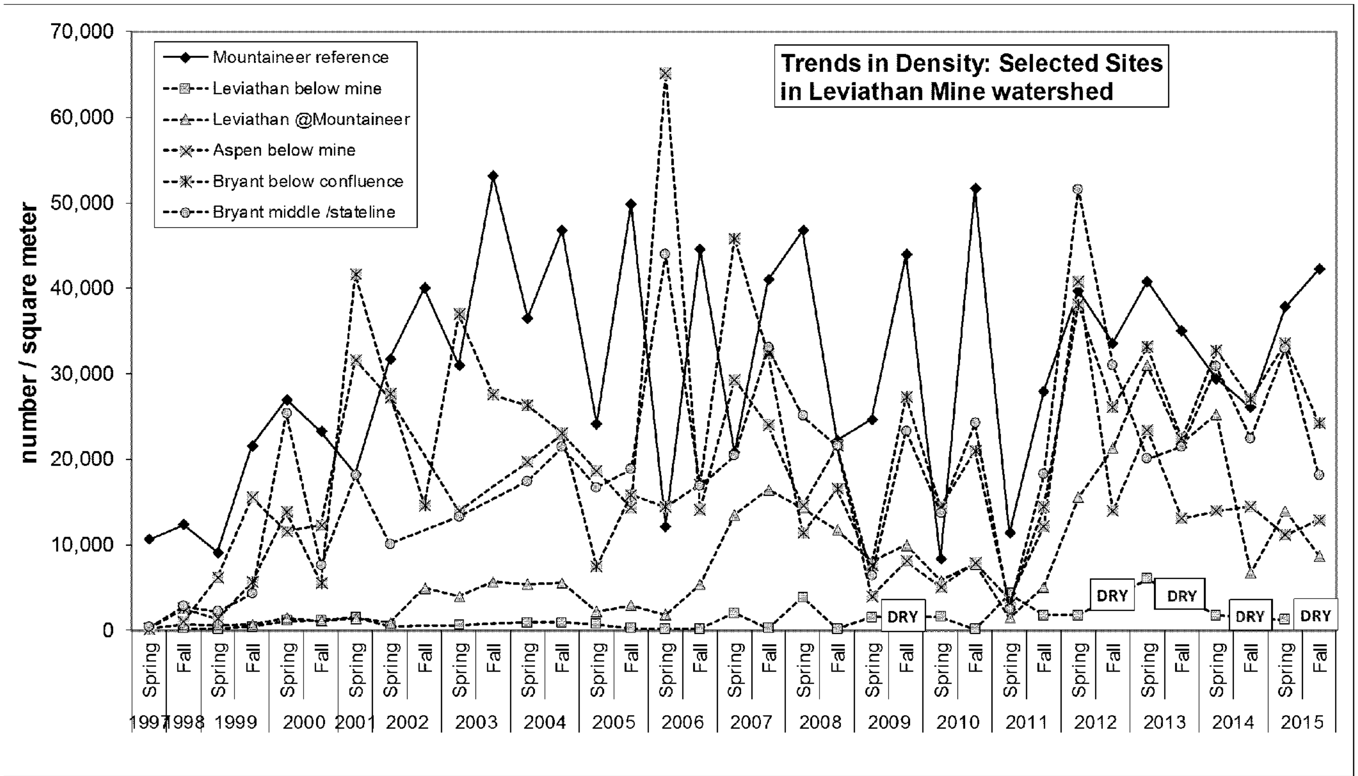

Figure 8. Average density (number / square meter) of total invertebrates from 5 replicate samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites.

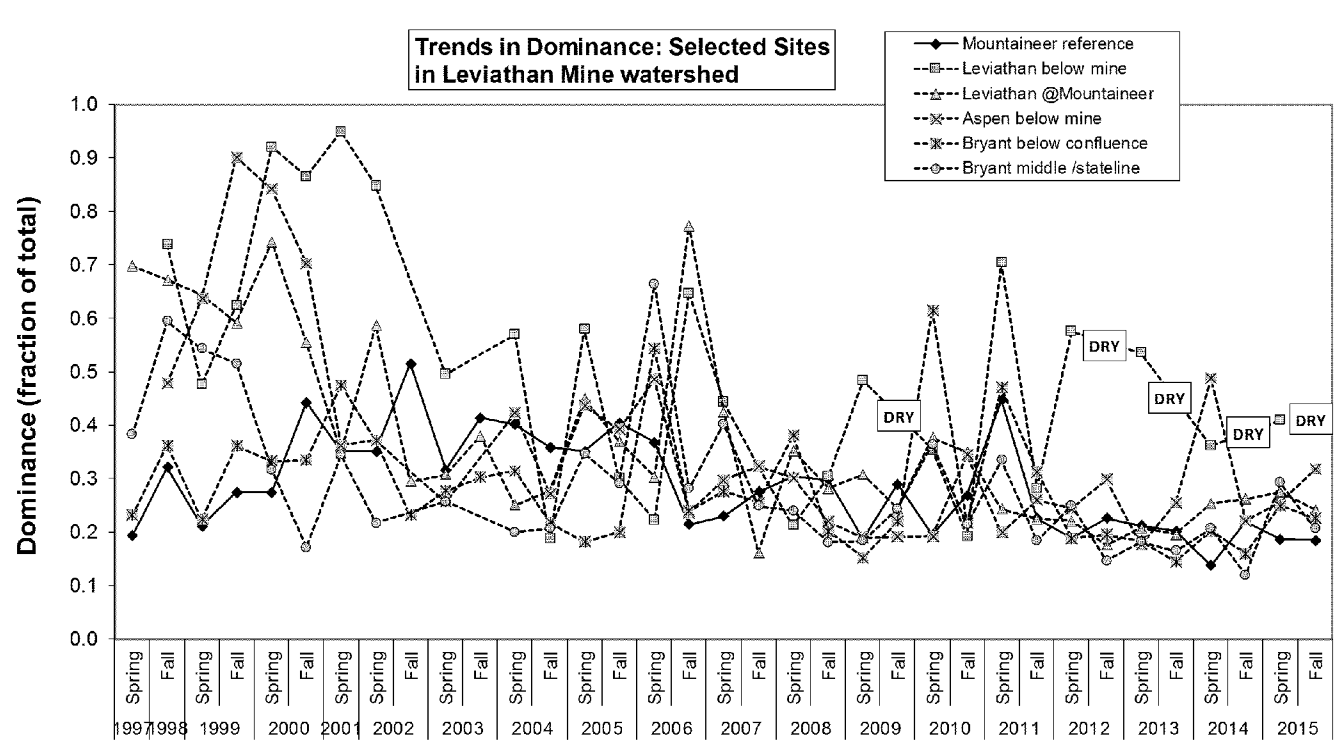

Figure 9. Average dominance of the most common invertebrate taxon from 5 replicate samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites.

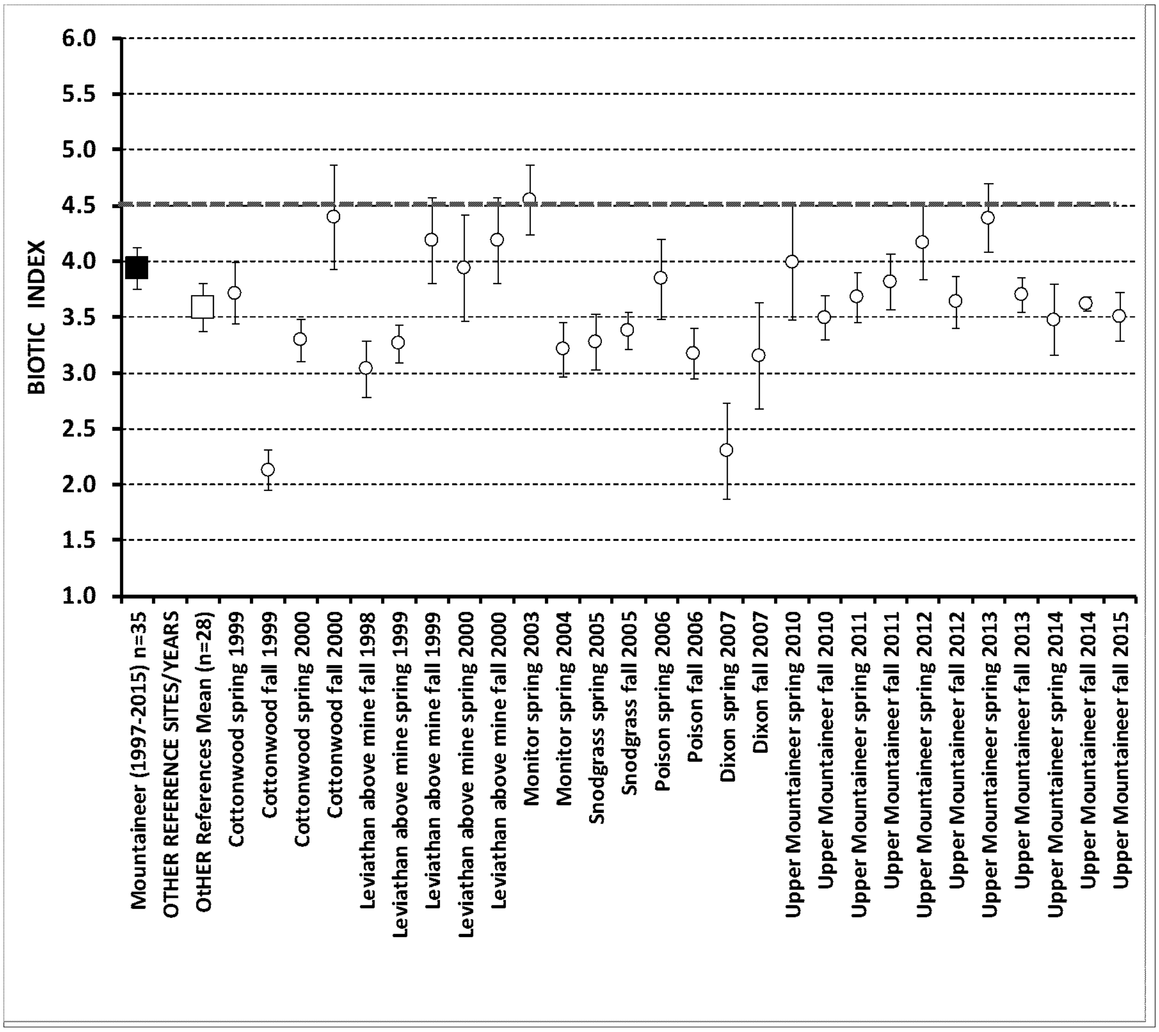

Figure 10. Biotic Index of reference streams from the Leviathan and nearby East Carson drainages contrasted to Mountaineer Creek. Large filled square symbol at left is the long-term mean for Mountaineer Creek from 1997-2015 (n=35) with the 95% confidence interval of the mean values. Open square symbol represents the long-term mean of all other reference site samples taken from n=28 surveys 1999-2015, with the 95% confidence interval of those means. Open circles show each reference site sample and standard deviations for n=5 replicates per site. The red line shows the range limit for these collective references, indicating study site means >4.5 can be considered impaired.

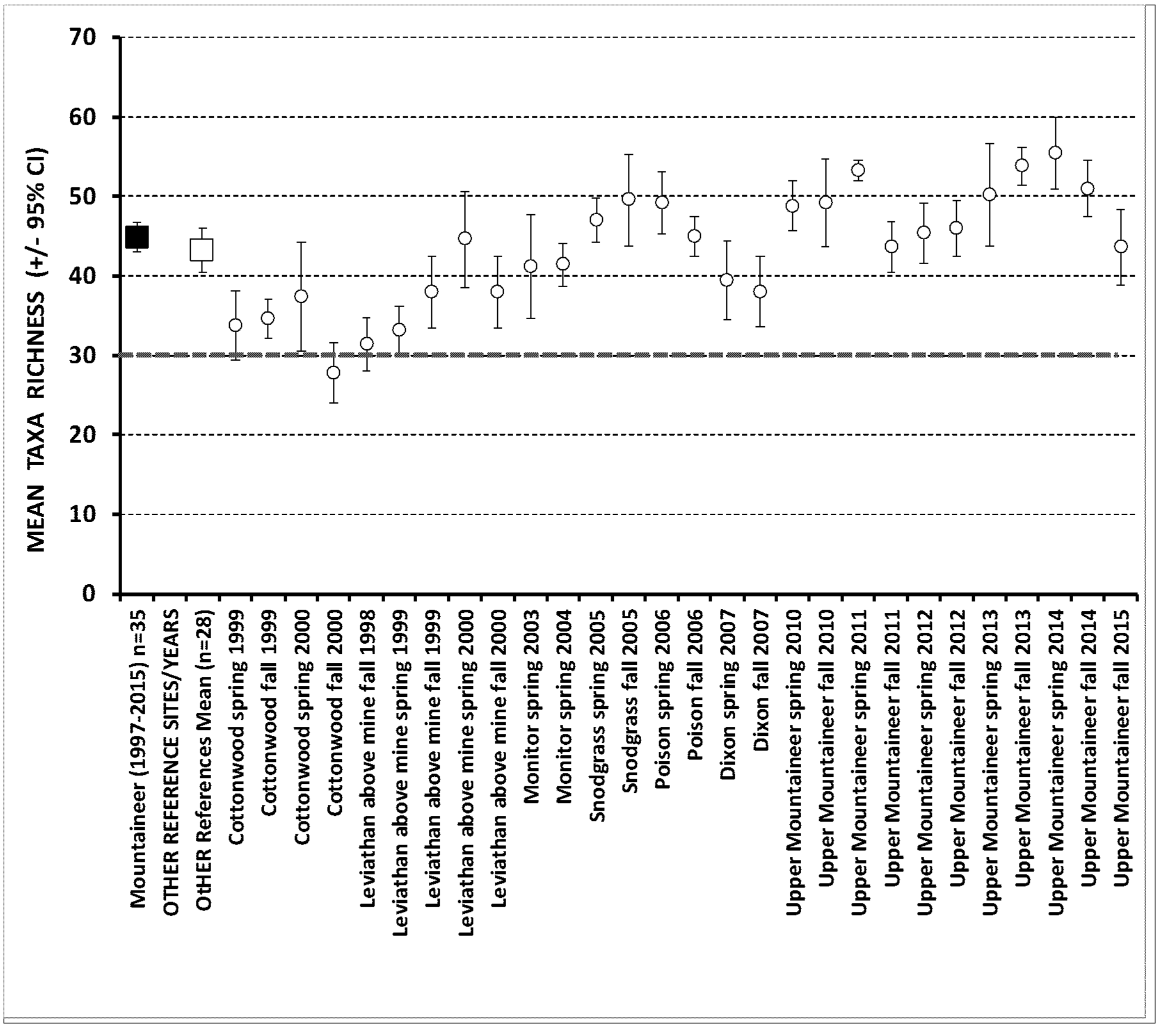

Figure 11. Mean taxa richness of reference streams from the Leviathan and nearby East Carson drainages contrasted to Mountaineer Creek. Large filled square symbol at left is the long-term mean for Mountaineer Creek from 1997-2015 (n=35) with the 95% confidence interval of the mean values. Open square symbol represents the long-term mean of all other reference site samples taken from n=28 surveys 1999-2015, with the 95% confidence interval of those means. Open circles show each reference site sample and standard deviations for n=5 replicates per site. The red line shows the range limit for these collective references, indicating study site means <30 can be considered impaired.

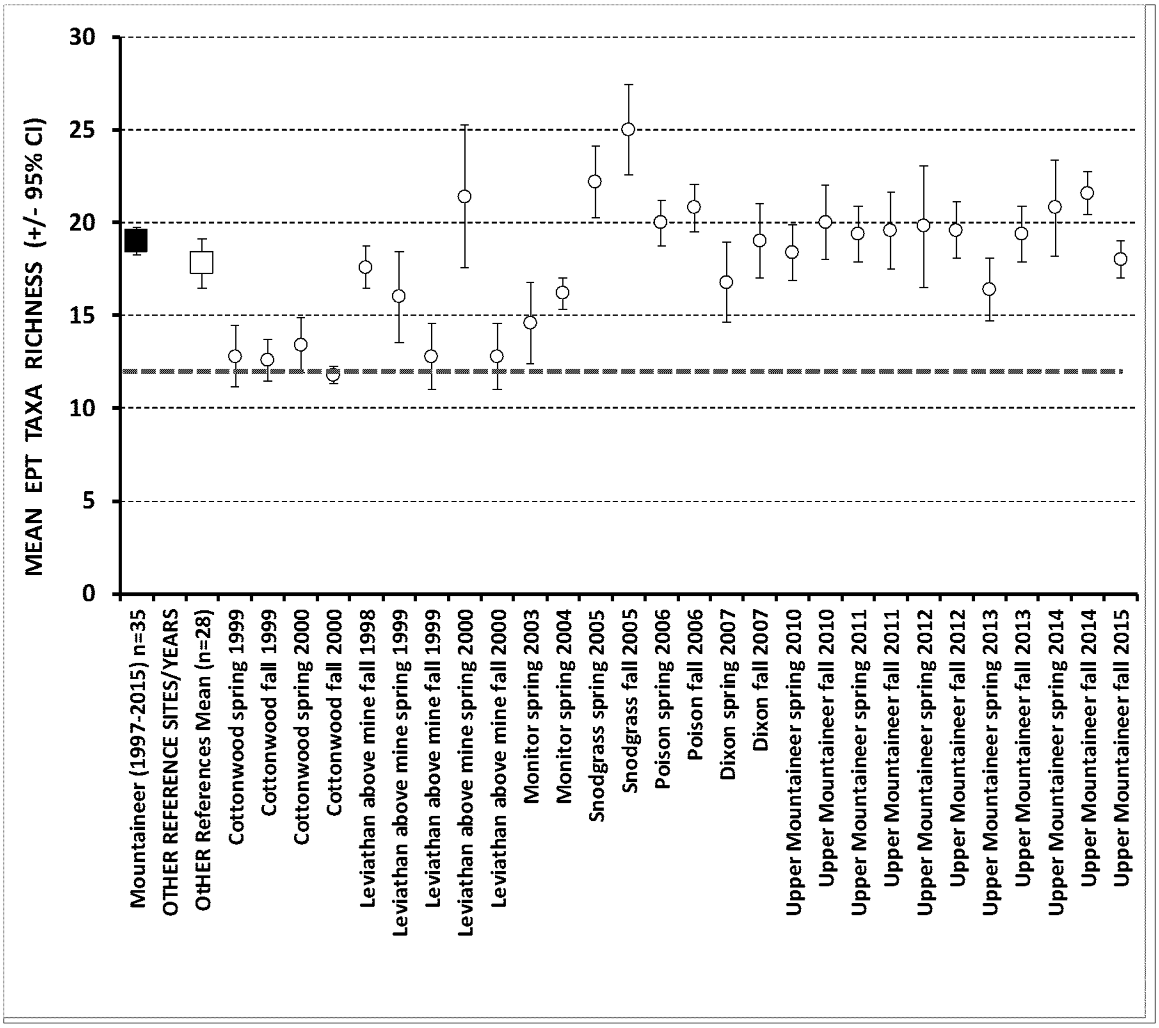

Figure 12. Mean EPT richness of reference streams from the Leviathan and nearby East Carson drainages contrasted to Mountaineer Creek. Large filled square symbol at left is the long-term mean for Mountaineer Creek from 1997-2015 (n=35) with the 95% confidence interval of the mean values. Open square symbol represents the long-term mean of all other reference site samples taken from n=28 surveys 1999-2015, with the 95% confidence interval of those means. Open circles show each reference site sample and standard deviations for n=5 replicates per site. The red line shows the range limit for these collective references, indicating study site means <12 can be considered impaired.

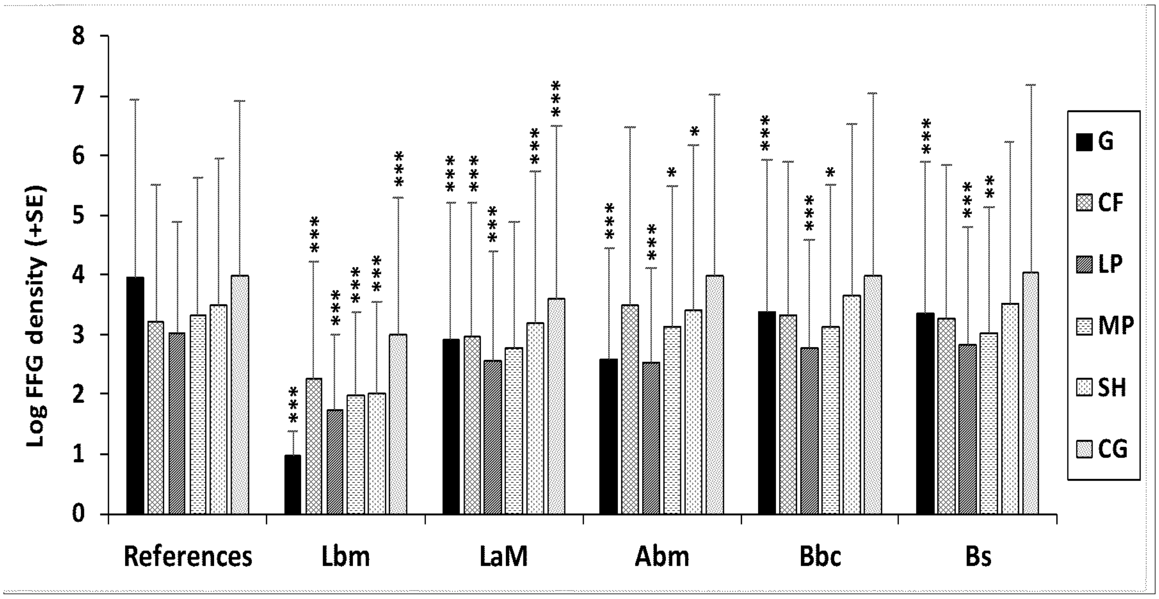

Figure 13. Mean log density of food web groups of aquatic invertebrates for Mountaineer and other reference sites compared to Leviathan/Aspen/Bryant creek sites exposed to AMD (+standard error bars), over 1998-2015 for each site. Also known as functional feeding groups, these show significant differences for algae-feeding grazers (G) and large predators (LP) across all sites compared to references, for all FFGs at Lbm and LaM, consumers of deposited (CG) and suspended organic matter particles (CF), did not differ at Abm or Bryant sites, predators (P), and those that shredders (SH) that feed on decomposing leaf and wood material also did not differ at Bryant sites (*p<0.05, **p<0.01, ***p<0.001, for paired t-tests corrected for multiple comparisons using Benjamini-Hochberg procedure and false discovery rate =0.05). Reference streams in this analysis include Mountaineer, Upper Mountaineer, Poison, Dixon and Leviathan Creek above the mine.

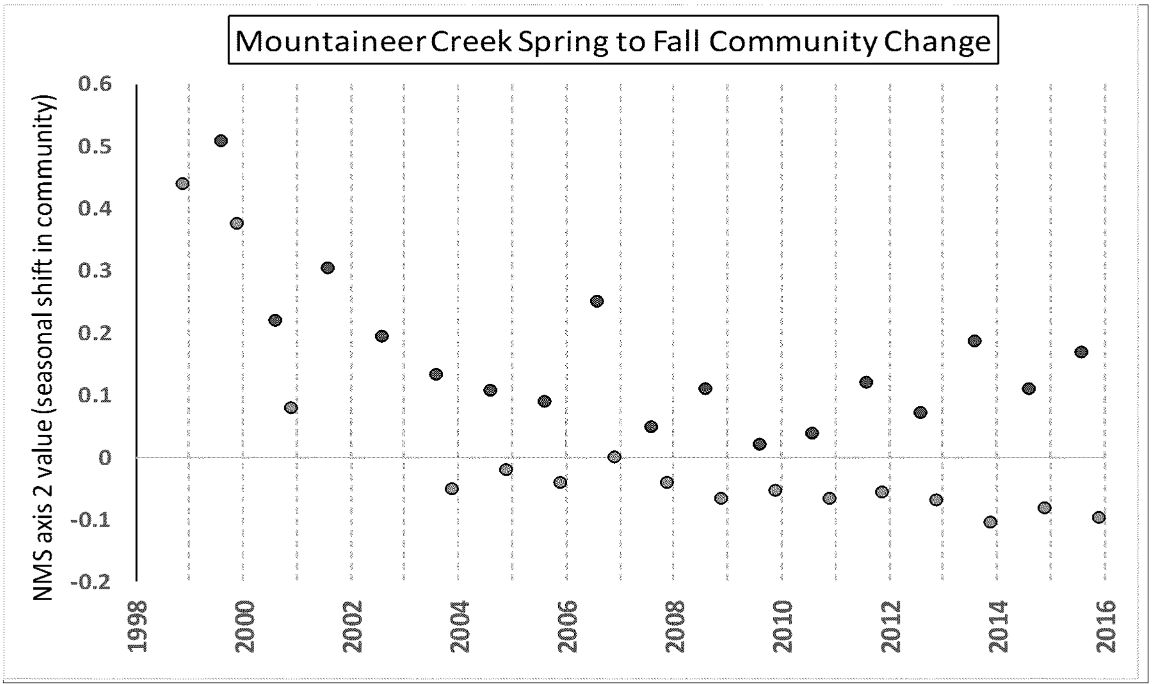

Figure 14. Seasonal change in community species composition indicated by differences within each year from spring (green) to fall (yellow). Note that last 3 years of drought (2013-205) and wet year 2006 show the greatest seasonal changes, suggesting the importance of hydrology as a factor in affecting stream life at this reference site, while the AMD sites are most affected by the influence of metals. The more separation there is between points on the NMS2 scale, the more different the species composition of the seasonal communities within each year.

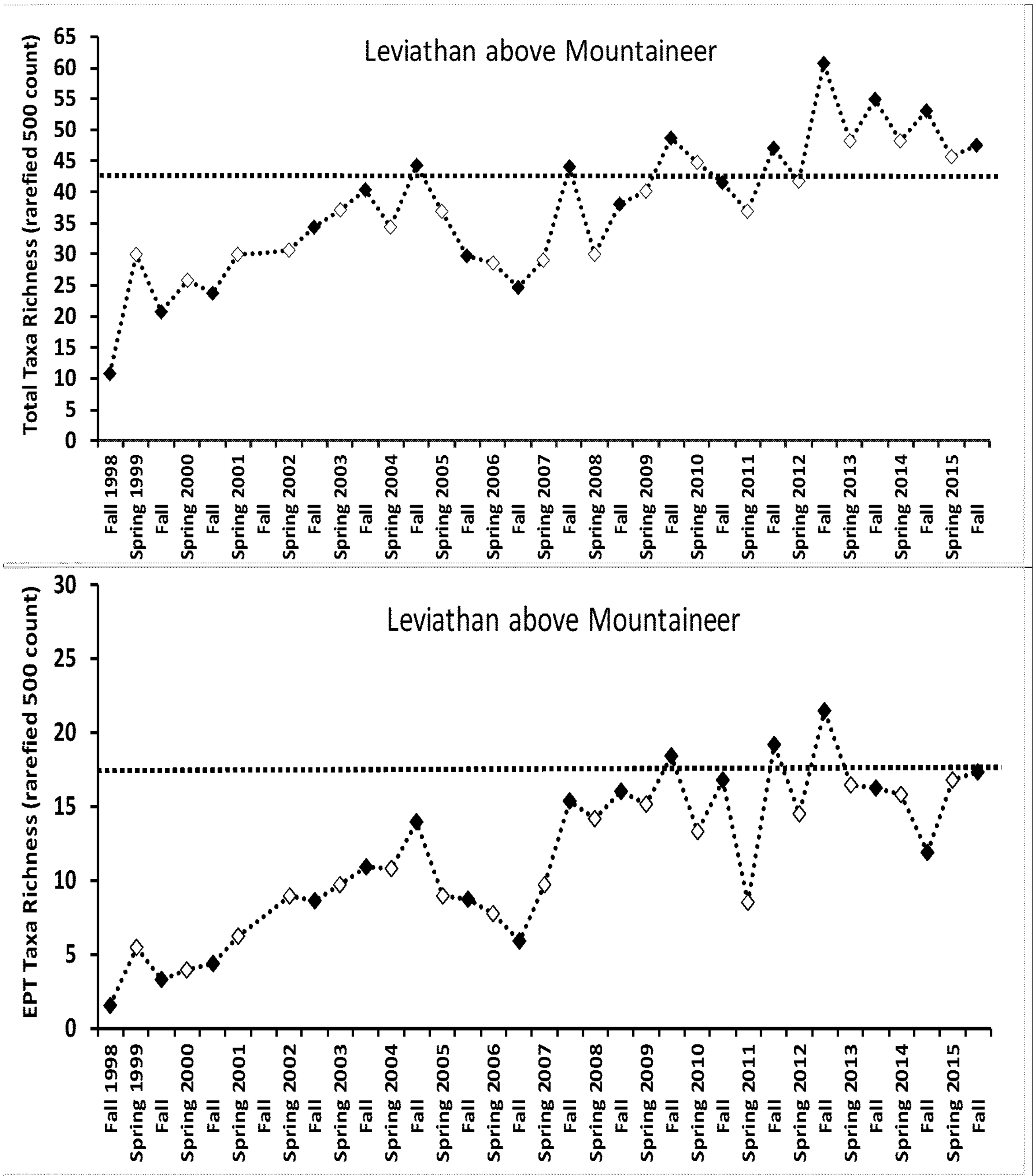

Figure 15. Seasonal relapse and recovery at Leviathan above Mountaineer. As an index location integrating Leviathan and Aspen AMD sources, and not diluted by Mountaineer Creek flows, this site shows recovery progress most clearly. Total richness (above) and EPT richness (below) improve in the fall (solid symbols) at the end of the treatment season, and relapse in the spring (open symbols) after a period of exposure to flows without active treatment. Dashed lines based on the 10^{th} percentile of reference site conditions. High runoff and drought periods can interfere with the seasonal pattern.